CELLULOSE ACETATE ELECTROPHORESIS
OF MALATE DEHYDROGENASE

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Various physicochemical techniques have been recently used to reveal the heterogeneity of the enzyme malate dehydrogenase (MDH). Thus two forms of the enzyme can be separated from each other by electrophoresis on starch gel, starch block, agar gel or ion-exchange chromatography, and the separated forms have been found to possess distinct characteristics (1-3). It appears that one form is associated with the supernatant fraction, while the other form with the mitochondria.

The present paper deals with an investigation of the electrophoretic mobility on cellulose acetate of the MDH's from rat and human tissues, together with some comparative studies on the supernatant and mitochondrial MDH's from rat liver, and the occurrence of the isozymes of MDH in the serum of normal rats and of those with experimental liver damages.

EXPERIMENTAL

The tissues studied were brain, heart, liver, muscle, pancreas, spleen, kidney and testis. They were homogenized in distilled water, frozen and thawed, and the particles were removed by high-speed centrifugation (for 30 minutes at 15,000 x g). The supernatant was subjected to electrophoresis on cellulose acetate paper at 4°C. Thereafter, the paper was placed into a glass dish of standard size, covered with 15 ml of substrate medium consisting of 1 vol. M malate, 1 vol. 10 mg/ml NAD+, 3 vol. 1 mg/ml nitro blue tetrazolium or 2-[(p-iodophenyl)-3-(p-nitropheryl)-5-phenyltetrazolium chloride and 0.3 vol. 1 mg/ml methyl phenazonium methosulphate, recommended by Barnett (4). The whole was incubated at 37°C in the dark for approximately 15 minutes, followed by washing with 5% acetic acid. If desired, densitometry can be carried out using a filter with the maximum transmission at about 540 m. Rat liver supernatant and mitochondrial MDH's were prepared by Hogeboom's method (5).

On the other hand, adult male rats were given carbon tetrachloride, 0.1 ml/100 g body weight, for producing experimental liver damages. The MDH activity of the serum samples was assayed by the staining intensities. In addition, the inhibitions by heating, trypsin and oxaloacetate of rat liver MDH were examined.
RESULTS

1. Electrophoretic Pattern

The separations were performed in a cold room at 4° for 90 minutes at 100 v/mA for each paper, 5.0 cm wide and about 5.0 cm long. The bands containing enzymic activity are visualized by the formation of a deep blue precipitate of formazan. Fig. 1 shows the MDH pattern obtained after electrophoresis at pH 8.6.

One band (MDH 1) was not far from the point of application, whereas the other band (MDH 2, migrating towards the cathode) was found far from the point. The ratio of the total MDH 1 activity to the total MDH 2 activity was about 1.5—2.0

Table 1 shows the percentages of the MDH isozymes in rat tissue homogenates. Each tissue had the two MDH's, and the ratio of its isozymes was also similar in various tissues.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MDH 1</th>
<th>MDH 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>67</td>
<td>33</td>
</tr>
<tr>
<td>Heart</td>
<td>51</td>
<td>49</td>
</tr>
<tr>
<td>Liver</td>
<td>62</td>
<td>38</td>
</tr>
<tr>
<td>Muscle</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Pancreas</td>
<td>58</td>
<td>47</td>
</tr>
<tr>
<td>Spleen</td>
<td>59</td>
<td>41</td>
</tr>
<tr>
<td>Kidney</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>Testis</td>
<td>65</td>
<td>35</td>
</tr>
</tbody>
</table>
The distribution of each band to the total MDH activity from human heart and liver is presented in Tab. II.

TABLE II

Percentage of Each Electrophoretically Separable Fraction to the Total MDH Activity from Human Heart and Liver
The figures show percentage as a mean of 5 cases

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MDH activity in Band</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Heart</td>
<td>56</td>
</tr>
<tr>
<td>Liver</td>
<td>53</td>
</tr>
</tbody>
</table>

Further, an aqueous extract of the acetone powder of rat and human tissues showed the existence of two MDH isozymes.

2. Comparison of Supernatant and Mitochondrial Malate Dehydrogenases in Rat Liver

It can be seen from Fig. 2 that the supernatant and mitochondrial MDH's from rats showed the similar pattern. However, analysis of the supernatant MDH showed a higher concentration of MDH 1 (relatively less cathodic mobility), whereas the mitochondrial MDH a higher concentration of MDH 2.

![Cellulose Acetate Electrophogram (90 min at pH 8.6)](image)

Mit., rat-liver mitochondrial MDH; Sup., rat-liver supernatant MDH.

It was suspended in the ratio of about 1.0 g of rat liver to 1.0 ml of 0.25 M sucrose for 30 minutes at 0°, and the supernatant was employed for electrophoresis. Two MDH bands were observed in the ratio of MDH 1 : MDH 2 about 3.0-4.0, suggesting the existence of two active components in the supernatant. This is in disagreement with the observations of Grimm (6) as well as of Thorne (3) that the supernatant MDH of several mammalian tissues differ from the corresponding mitochondrial MDH.

3. Attempts to Alter the Pattern of Bands

Rat liver MDH isozymes were treated in a number of attempts to alter the pattern of bands observed after electrophoresis at pH 8.6. As measured by the staining intensities, the two bands were almost completely inhibited by heating (at 60° for 10 minutes) and oxaloacetate (final concentration, $10^{-4} M$), but partially inhibited by trypsin treatment (1000 units supplied from Motida Corporation,
incubated for 30 minutes at 37°C), especially in the case of MDH 2. (Fig. 3)

![Fig. 3 Inhibitory Effects of Trypsin and Temperature on the MDH Isozymes of Rat Liver Extracts](image)

4. Cellulose Acetate Electrophoresis of Rat Serum

Incubation of MDH 1 and MDH 2 (purified partially from the extracts of acetone powder of rat liver), with the equal amounts of normal rat serum at 37°C for 1 hour before electrophoresis did not affect the mobility of the isozymes.

After 48 hours, adult male rats, given carbon tetrachloride, were killed by exsanguination under anaesthesia. The blood was allowed to clot and the serum removed after centrifugation. The MDH activity of the serum was assayed by the method of Barnett (4), in order to investigate the presence of the isozymes of MDH in serum. (Fig. 4)

![Fig. 4 Localisation of MDH Isozymes in Cellulose Acetate after Electrophoresis](image)

The results (Fig. 4) show that the MDH 2 has the electrophoretic mobility of γ-globulin, and the MDH 1 a mobility intermediate between α2- and β-globulins. In the serum from normal rats, only weak MDH 1 was present. Very strong bands of MDH 1 were present in the serum of the rats 48 hours after carbon
tetrachloride treatment. Much weaker bands of MDH 2 were also detectable in the serum of these rats. It was concluded that the increased serum MDH following experimental liver damages in rats is mainly in the form of MDH 1.

**DISCUSSION**

Thorne, Grossman and Kaplan demonstrated the presence of six active components by starch gel electrophoresis of a preparation of horse-heart mitochondrial MDH.

In the present work, electrophoretic heterogeneity of MDH on cellulose acetate was observed in the materials from different rat organs. Similar electrophoretic bands were obtained from the homogenates of human livers and hearts. A number of papers have recently reported that two or more separable forms of MDH occur in the extracts of a variety of tissues (1-3, 6-8), and that they were of mitochondrial and supernatant origin.

However, our electrophoresis on cellulose acetate demonstrated the presence of two active components; similar electrophoretic patterns were obtained from both the supernatant and mitochondrial MDH’s of rat livers, but it was shown that the ratio of total MDH 1 activity to the total MDH 2 activity from the supernatant enzymes was somewhat different from the mitochondrial enzymes.

Boyd (9) showed that the increased glutamic-oxaloacetic transaminase (GOT) following liver damage is mainly in the form of supernatant GOT. Our findings of MDH isozymes are essentially similar to those of Boyd, i.e., more release of soluble enzyme (MDH 1) from damaged cells would be expected. The immunological approach to detect the similarities of the enzyme has also attracted much interest, and the immunological heterogeneity of MDH’s will be able to be used to test the release into the serum. Further studies on these problems are necessary. The significance of the presence of the two enzymes having seemingly the same activity remains to be elucidated.

**SUMMARY**

1. Heterogeneity of malate dehydrogenase (MDH) activities was proved by electrophoresis on cellulose acetate of rat and human tissue homogenates. Two or more separable forms of MDH activities were found in these tissues. Each tissue exhibited a similar distribution of MDH activities.

2. The supernatant MDH showed a band of relatively less cathodic mobility (MDH 1), but the mitochondrial MDH (MDH 2) was found to be far more mobile, migrating toward the cathode. However, two MDH bands were observed in both fractions.

3. The acetone powder extracts showed also the two MDH activities.

4. The enzymes isolated from rat liver extracts were easily inhibited by heating, trypsin and oxaloacetate according to the staining methods.

5. It was concluded that the increased serum MDH following experimental liver damage in rats is mainly the form of MDH 1.
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REFERENCES